Recent Advances in Insect Virology[1]

Y. TANADA
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

I wish to express my mahalo to Dr. Minoru Tamashiro, Dr. Frank Haramoto and other members of the program committee for this invitation and for making it possible to return to Hawaii.

When a speaker is provided the freedom to select and review a subject, he is under great temptation to stress areas of his expertise. I have yielded to this temptation. However, I hope to be excused because I shall discuss the studies made by my students and myself with insects found in Hawaii, and especially with viruses that originally came from Hawaii.

Insect virology has progressed extensively during the past decade, and I shall discuss mainly the progress made during this period. Advances have occurred in biochemistry, molecular biology, cytopathology, pathophysiology, host susceptibility, new forms of viruses and strains, microbial control, and epizootiology. Since I do not have time to review all of these aspects, I shall confine myself to the following seven areas: (1) types of insect viruses, (2) virus strains and the basis for their separation, (3) mode of virus invasion through the midgut, (4) interactions of viruses, (5) virus toxin, (6) use of virus in microbial control, and (7) epizootiology of virus diseases.

I have listed the general types of viruses that occur in insects (Table 1). I have excluded viruses that also infect the arthropod vectors of plant and animal viruses, although these viruses cause pathologies in the arthropods. Up to a decade ago, the most common types were the nuclear- and cytoplasmic-polyhedrosis and the granulosis viruses. These three viruses attack primarily Lepidoptera, but the nuclear-polyhedrosis viruses also commonly infect a group of hymenopterous insects, the sawflies. Recently, other orders of insects have been found susceptible to the polyhedrosis viruses. The sigma virus of *Drosophila* and the sacbrood virus of honeybee have also been known for many years.

During the past decade, a large number of nonoccluded viruses, that are similar to the sacbrood virus, have been reported from different insects, such as the honeybee, termite, grasshopper, moth, beetle, and mite. Other recent discoveries are the iridescent virus (Xeros, 1954), the rhabdionvirus

[1]Invitational paper presented at the 54th Annual Meeting of the Pacific Branch Entomological Society of America in June 1970 in Honolulu, Hawaii.

TABLE 1. *Types of Insect Viruses*

Type	*Inclusion Body*	*Nucleic Acid*	*Host*
*Nuclear polyhedrosis virus	Polyhedra	DNA	Lepidoptera, Hymenoptera, Diptera, Neuroptera
*Cytoplasmic polyhedrosis virus	Polyhedra	RNA	Lepidoptera, Diptera, Hymenoptera, Neuroptera
*Granulosis virus	Capsule	DNA	Lepidoptera
Poxvirus	Spherule	DNA	Lepidoptera, Coleoptera, Orthoptera
*Paillotiavirus	Polymorphic	?	Lepidoptera
Moratorvirus (*sacbrood, paralysis, flacherie, foulbrood)	None	RNA	Hymenoptera, Isoptera, Orthoptera, Lepidoptera, Coleoptera, Acarina
*Sigma virus	None	RNA	Diptera
Iridescent virus	None	DNA	Diptera, Lepidoptera, Coleoptera
Rhabdionvirus	None(?)	DNA	Coleoptera
Densonucleosisvirus	None	DNA	Lepidoptera

*Viruses known for over two decades.

(Huger, 1966), the poxvirus (Vago, 1963), and densonucleosisvirus (Vago, *et al.*, 1964).

The iridescent virus is a cubic virus, icosahedral in outline and produces, when viewed by reflected light, an iridescent color caused by the crystallization of the virus particles. In transmitted light, its color is amber to orange. These very interesting and optically beautiful viruses were first reported from the cranefly, *Tipula paludosa*, by Xeros (1954). The iridescense is also visible in the tissues of a severely infected insect. These viruses have also been found in Coleoptera and Lepidoptera.

An unusual virus which appears to exist in spherical to rod-shaped forms was found in the rhinoceros beetle, *Oryctes rhinoceros*, by Huger (1966), who called it the rhabdionvirus. This is the first virus that has been tested and found to be highly promising for the control of a beetle, *Oryctes rhinoceros* (Marschall, 1970).

Insect virus, very similar in structure and characteristic to the poxvirus of vertebrates, has been found in Coleoptera (Vago, 1963; Vago *et al.*, 1968) and Lepidoptera (Meynadier *et al.*, 1968; Roberts and Granados, 1968). The densonucleosisvirus is a DNA virus isolated from Lepidoptera (Vago *et al.*, 1964), and it resembles the papilloma virus, which causes warts in man.

Strains of Insect Viruses

Over twenty years ago, there was little attempt to differentiate strains of insect viruses, primarily because of limited study, and because of the lack of a sound basis to designate strains. Gershenson, in 1959, first observed that the shapes and sizes of the inclusion bodies of the nuclear-polyhedrosis virus were determined exclusively by the virus and not by the host. He isolated mutants with distinctly-shaped inclusion bodies.

In the cytoplasmic-polyhedrosis viruses, strains have also been separated by the shapes of the inclusion body. Professor Aruga and his colleagues (1961) have isolated strains with tetragonal (cuboidal) and hexagonal (icosahedral) polyhedra. In the granulosis virus, strains which form cubic inclusion bodies rather than the typical capsules have been isolated from the spruce budworm (Stairs, 1964a), and the codling moth (Stairs *et al.*, 1966).

Virus strains have been designated on the basis of virulence and pathogenicity. Strains, which differ in virulence, may occur in nuclear-polyhedrosis viruses from different localities or from different host species. Iridescent viruses may be related but differ in their pathogenicities.

The sigma virus of *Drosophila* has been most extensively studied in multicellular animals from the standpoint of genetics, and numerous genetic variations of this virus have been found (see Seecoff, 1968).

Serological and biochemical methods have been applied to establish relationships among insect viruses (Aleksandrov, 1965; Hukuhara and Hashimoto, 1966; Bellett and Inman, 1967; Kalmakoff and Tremaine, 1968; Cunningham and Longworth, 1968; Krywienczyk *et al.*, 1969).

Strains may express different pathologies within the host and may also possess different biological properties, such as a synergistic effect. Paillot described three virus diseases, pseudo-grasserie 1, 2 and 3, in a single insect species, the cutworm (Paillot, 1934; 1935; 1937). However, it is questionable whether these 3 granulosis-virus infections are caused by three strains or by three different types of viruses. In the mosquitoes, there are strains of iridescent viruses that produce different colors in the larva (Chapman, *et al.*, 1966; Woodard and Chapman, 1968).

In 1966 when we renewed our study on synergism, we found that the granulosis virus from Hawaii was contaminated with a cytoplasmic-polyhedrosis virus. Consequently, we obtained a clean sample from Oregon through Dr. G. Wittig. This Oregon strain, much to our surprise, did not enhance the infection of the nuclear-polyhedrosis virus in the armyworm larvae. This was very embarrassing, for it meant that all of our previous studies on synergism were incorrect. Although I was "shook up", I retested the Hawaii granulosis virus. The virus was first purified to remove the contaminating cytoplasmic-polyhedrosis virus. When the two strains were tested, the Hawaii isolate had a synergistic effect on the nuclear-polyhedrosis virus, while the Oregon isolate did not. Thus, much to my relief, there were two strains of the granulosis virus that produced similar pathologies, but differed in their synergistic properties (Tanada and Hukuhara, 1968).

The above difficulty suggested that we should also examine the nuclear-polyhedrosis virus collected from Hawaii in 1958 to 1960. Out of the inocula prepared from 17 larval specimens, only one inoculum produced the typical symptoms of nuclear-polyhedrosis virus infection. The

remaining inocula caused different signs and symptoms, such as, a yellow brown coloration rather than the typical pinkish white, the larvae took much longer to die, their tracheal and hypodermal cells were greatly hypertrophied, and the fat cells were not infected (Tanada *et al.*, 1969).

Although I have described several criteria by which strains have been designated for insect viruses, are these adequate for the establishment of strains? This would depend upon what we mean by a virus strain, or even a virus species! At present, we are not able to answer these questions.

Path of Virus Invasion Through the Midgut

A virus most commonly infects an insect through the mouth, into the midgut, and into the internal tissues. However, the actual mode of invasion of the virus through the midgut epithelium and into the hemocoel remained obscure until about two years ago. In 1968, Harrap and Robertson observed that a nuclear-polyhedrosis virus infected and developed in a few columnar midgut cells of the larva, and that some virus particles were found in the basement membrane. They concluded that the virus was first produced in the cells and then passed through the basement membrane into the hemocoel. Summers, in 1969, observed granulosis-virus particles in the microvilli of midgut cells within 2 hours after the virus had been fed to cabbage-looper larva. He also observed virus multiplication in the nuclei of midgut cells, and the presence of complete rods, some of which were contained within vesicles, in the cytoplasm. He speculated that the virus rods within the vesicles passed through the basement membrane and into the hemocoel.

Apparently about the same time that Summers was conducting his study, Ruth Leutenegger and I (1970) were also investigating a granulosis virus in the same host insect. Our results were essentially similar to those of Summers, but we did not find virus particles in the microvilli. However, we made additional observations that virus particles occurred in the intercellular spaces, in possibly the endoplasmic reticulum, and in the basement membrane. We have postulated two methods of virus invasion into the larval hemocoel after the virus particles had been freed from the inclusion bodies in the gut lumen. In the first method, the virus rods enter the interecellular space between two columnar cells and then into the basement membrane, and into the hemocoel. In the second method, the virus enters and multiplies in the nucleus of a midgut cell. The virus rods then pass into the cytoplasm, through the basement membrane and into the hemocoel. In either case, the passage of the virus through the basement membrane takes place as complete particles.

Interaction of Viruses Within the Insect's Body

There are numerous examples of mixed virus infections occurring in a single host insect. In only a few cases were the interactions of the

viruses carefully examined, and in some of these cases, interference and synergism were observed. Interference was reported when the insect larvae were fed different strains and types of insect viruses (Bird, 1959; 1969; Aruga *et al.*, 1961; Tanada *et al.*, 1969). Such interference occurred even when the interfering virus was inactivated by heat or ultraviolet light (Aruga *et al.*, 1961; 1962; 1963a, b; Aruga and Hashimoto, 1965).

I first observed synergism between a granulosis and a nuclear-polyhedrosis virus in the armyworm in 1956, while on the staff of the University of Hawaii. Since this study is familiar to some of you, I shall summarize briefly the results. The young larvae (first and second instars) were found highly susceptible to the two viruses. The older larvae were resistant when fed the viruses separately, but became highly susceptible when fed the viruses together, a synergistic interaction. Further studies in 1959, revealed that the granulosis virus was the synergist or activator for the nuclear-polyhedrosis virus, and the granulosis virus retained the enhancing capacity even when inactivated by heat.

In 1967, Dr. Hukuhara and I investigated the nature and characteristic of the enhancing factor, the site of its activity, and its mode of action. There was a direct increase in the number of larvae infected with the nuclear-polyhedrosis virus as the concentrations of the capsules of the granulosis virus increased (Tanada and Hukuhara, 1971). The granulosis virus increased the infectivity of the nuclear-polyhedrosis virus by as much as 10,000 times.

The evidence, therefore, is strong that the capsules enhance the infection of the nuclear-polyhedrosis virus, but the question arises, "Where is the enhancing activity located in the capsules?" The capsule protein was separated from the virus particle by treatment with alkali and was found to enhance the nuclear-polyhedrosis virus. Thus the active factor occurred in the capsule protein rather than in the virus rod. By means of column chromatography and acrylamide-gel electrophoresis, we found that the active fraction was not the major component of the capsule protein (Tanada and Watanabe, 1971). The site of action of this synergistic factor appeared to be in the midgut and not in the hemocoel.

Virus Toxins

I have found working with the Hawaii viruses very exciting and most interesting, and after our unusual findings on strains and on synergism, I did not expect any further rewards from them. But they seemed to have been blessed by the Kahuna. One of my students, Harry Kaya (1970) has made a very important discovery with the Hawaii viruses. He found that, in virus-infected armyworm larvae, a toxic factor was produced that killed the internal parasite, *Apanteles militaris*. This discovery, by Harry, is apparently the first record of a toxin formed in virus infections in invertebrates.

TABLE 2. *Partial list of insects and mite which have been controlled with virus in field experiments during* 1960 *to* 1970

Order	*Species*	*Type of virus*	*Reference*
Lepidoptera	*Adoxophyes reticulana*	Nuclear-polyhedrosis virus; cytoplasmic-polyhedrosis virus	Ponsen and de Jong, 1964
	Agrotis segetum	Granulosis virus	Dikasova, 1964
	Choristoneura fumiferana	Nuclear-polyhedrosis virus; granulosis virus	Stairs and Bird, 1962
	Dendrolimus sibiricus	Granulosis virus	Luk'yanchikov, 1964; Poltev, 1964
	Dendrolimus spectabilis	Cytoplasmic-polyhedrosis virus	Koyama and Katagiri, 1968; Katagiri, 1969
	Heliothis virescens	Nuclear-polyhedrosis virus	Allen *et al.*, 1967b; Montoya *et al.*, 1966
	Heliothis zea	Nuclear-polyhedrosis virus	Wolfenbarger, 1964; Ignoffo *et al.*, 1965; Falcon *et al.*, 1965; Allen *et al.*, 1967a,b; Montoya *et al.*, 1966; Young and Ham, 1m966; Chapman and Bell, 1967; Oatman *et al.*, 1970
	Hyphantria cunea	Nuclear-polyhedrosis virus; granulosis virus	Oliver, 1964
	Kotochalia junodi	Nuclear-polyhedrosis virus	Ossowski, 1962
	Laspeyresia pomonella	Granulosis virus	Falcon *et al.*, 1968
	Malacosoma disstria	Nuclear-polyhedrosis virus	Stairs, 1964b; 1965
	Malacosoma fragile	Nuclear-polyhedrosis virus	Stelzer, 1965; 1967
	Mamestria brassicae	Nuclear-polyhedrosis virus	Akutsu, 1968
	Oraesia emarginata	Cytoplasmic-polyhedrosis virus	Oho, 1966
	Orgyia pseudotsugata	Nuclear-polyhedrosis virus	Morris, 1963

Order	Species	Type of virus	References
	Pieris rapae	Granulosis virus	Wilson, 1960; Fox and Jaques, 1966; Akutsu, 1968
	Porthetria dispar	Nuclear-polyhedrosis virus	Rollinson *et al.*, 1965; Magnoler, 1968
	Porthetria fumida	Nuclear-polyhedrosis virus; cytoplasmic-polyhedrosis virus	Koyama and Katagiri, 1967; Katagiri, 1969
	Spodoptera frugiperda	Nuclear-polyhedrosis virus	Young and Hamm, 1966
	Thaumetopoea pityocampa	Nuclear-polyhedrosis virus; cytoplasmic-polyhedrosis virus	Grison, 1960; Sidor, 1965
	Trichoplusia ni	Nuclear-polyhedrosis virus	Genung, 1960; Elmore, 1961; Semel, 1961; Hofmaster and Ditman, 1961; Getzen, 1962; Ignoffo, 1964; Wolfenbarger, 1965; Woodall and Ditman, 1967
Hymenoptera	*Diprion hercyniae*	Nuclear-polyhedrosis virus	Bird and Burk, 1961
	Neodiprion pratti pratti	Nuclear-polyhedrosis virus	McIntyre and Dutky, 1961
	Neodiprion sertifer	Nuclear-polyhedrosis virus	Rivers, 1964; Sidor and Nikolić, 1966
	Neodiprion swainei	Nuclear-polyhedrosis virus	Smirnoff, 1961; Smirnoff *et al.*, 1962; Smirnoff, 1964
Coleoptera	*Oryctes rhinoceros*	Rhabdionvirus	Marschall, 1970
Acarina	*Panonychus citri*	Moratorvirus	Gilmore, 1965; Shaw *et al.*, 1968a,b; Putman, 1970

I would have preferred to have Harry relate to you his discovery, which is a landmark in insect virology. However, I hope that some day you will permit him to describe his studies in detail.

Microbial Control

During the past decade, there has been a great increase in the number of field tests with viruses that resulted in satisfactory or promising control of insects. The list presented in Table 2 is incomplete, and does not include insects, such as the alfalfa caterpillar, that had been tested prior to the past decade, but it includes those that had been retested. The increase is not too surprising because about 300 different viruses have been described in over 250 insect species (Ignoffo, 1968). Thus, many more viruses await field testing.

Since there is no time to discuss all of the examples, I shall confine myself only to the control of the corn earworm, *Heliothis zea*, with a nuclear-polyhedrosis virus. This insect has been most thoroughly investigated as far as microbial control with virus is concerned. The methodology and technology of the use of the virus have been worked out, and the virus has been found effective under field conditions by many workers. Its virulence and pathogenicity for *Heliothis zea* have been established; methods have been developed for mass propagation; field application techniques have been developed, including the use of the virus with attractants, adjuvants, and chemical insecticides; and this virus has been found harmless to vertebrates (see Ignoffo, 1968).

I shall discuss the control of *Heliothis* on two crops: sweet corn and cotton. When I transferred to California, I selected as a project the microbial control of *Heliothis* on sweet corn, because this would offer a severe test on the effectiveness of pathogens for the control of insects of low economic threshold. Some of my colleagues greatly doubted my judgment. They predicted a failure since they believed that pathogens took too long to kill an insect, such as the corn earworm, where a single larva may cause damage before dying from the pathogen. However, they did not realized that certain pathogens, when applied to first- and second-instar larvae, can kill an insect within 1–2 days, almost as fast as some chemical insecticides. Moreover, in some cases, the insects stop feeding shortly after ingesting the pathogen.

Our studies showed that the virus was nearly as effective as the standard insecticide, DDT (Tanada and Reiner, 1962). The criterion that we used as the basis for control was that an ear was considered damaged if a single kernel was eaten. Our study was apparently the first favorable attempt at controlling *Heliothis zea* on sweet corn with pathogens.

Other workers, however, reported unsatisfactory results. Anderson *et al.* (1963) used the same severe criterion that we had used, and obtained results in Southern California that showed the virus to be inferior to DDT. Recently, Oatman *et al.* (1970) working under similar conditions in South-

ern California, used a less severe criterion for control based on "marketable ears" (i.e., less than 25% of the ear from the tip injured by larval feeding) as defined by the Agricultural Code of California. Their results were: 77–87% marketable ears with virus sprays, and 91–100% marketable ears with virus dust.

The control of corn earworm on cotton with the virus has been conducted in several states, such as California, Mississippi, and Texas. Generally, the reports have been very favorable, and in some cases better control has been obtained than with the standard insecticides, usually DDT-Toxaphene. Several workers, such as Falcon *et al.* (1965), Allen *et al.* (1967a), Fernandez *et al.* (1969) have attempted to integrate the virus into the pest control program of cotton.

The future of viruses for insect control is very promising but, at present, there are two major obstacles: (1) no virus has yet been approved for commercial applications by FDA, and (2) the problem of mass production and the cost of the virus to the farmers.

Epizootiology

Some significant progress has taken place in the interaction of insect populations and viruses. I shall confine myself to two aspects: (1) the interrelation between virus and host density, and (2) the persistence of viruses in the soil habitat.

The general belief is that virus epizootics develop spontaneously with a very high host density. This belief is not completely correct because studies, prior to the past decade, with the sawfly by Bird and Elgee (1957), and our studies with the armyworm (Tanada, 1961), showed that virus epizootics generally begin from a low incidence that increases as the host density increases; a typical density dependent factor. Recently, this has been shown with the nuclear-polyhedrosis viruses and populations of the eye-spotted bud moth (Jaques and Stultz, 1966), and the gypsy moth (Doane, 1970).

The most extensive study on virus epizootics has been conducted during the past decade with a nonoccluded virus and the citrus red mite, *Panonychus citri*. Gilmore and Munger (1965) reported that, in laboratory tests, the incidence of virus infection in the citrus red mite increased progressively with host density. In the field, Shaw *et al.* (1968b) found low mite populations with a low incidence of virus infections (enzootic). The high incidence of disease was correlated with high mite density, and invariably, epizootics developed in large populations.

During the past five years, we have sampled in alfalfa fields the populations of the beet armyworm, *Spodoptera exigua*, the alfalfa caterpillar, *Colias eurytheme*, the alfalfa looper, *Autographa californica*, and the armyworm, *Pseudaletia unipuncta*. We have found that virus-infected larvae occur even at a very low host density. This is especially true of the alfalfa

looper during the spring months, when less than one larva per alfalfa clump is present, yet over 50% of the larvae are infected with virus. In the beet armyworm, which is the most abundant Lepidoptera in our studies, the incidence of virus infection increases with a rise in host density.

Thus, evidence is accumulating that a virus epizootic usually begins with a low incidence of virus infection in the population. The question arises, how is the virus infection initiated in a population of low density? I shall now discuss one of the possible sources from which the virus may gain access to an insect population. This is the soil habitat.

The first demonstration that virus persisted in the soil was made by Thompson and Steinhaus in 1950 with the nuclear-polyhedrosis virus of the alfalfa caterpillar. An increasing number of workers have made similar observations (Jaques, 1964; 1967; 1969; 1970a, b; David, 1965). Jaques observed that the viruses of the cabbage looper and the imported cabbageworm did not diminish appreciably in the soil surface. About 25% of the original viruses that he applied were still present after 5 years. He reported that the viruses, applied to the soil, protected the plants against the cabbage looper and the cabbageworm much longer than the virus, which had been sprayed on the plants.

During the past three years, we have tested the soils in alfalfa fields to determine whether they contained viruses during different periods of the year, especially after the winter months when rainfall is the highest and when the plants usually wither to the ground. The soils were fed to the alfalfa caterpillar, beet armyworm, alfalfa looper, armyworm, and cabbage looper. We have found that several viruses and a microsporidian are able to persist in the field soil.

Conclusion

In conclusion, I wish to remind you that I have touched upon, only briefly, a portion of the recent advances in insect virology. Although insect virology has not yet attained the levels of plant and vertebrate virology, its progress and development have been very significant during the past decade, and this science has attained a modicum of sophistication and has attracted the attention of other virologists, including molecular biologists. I visualize that in the coming decade, insect viruses will play a very important role, not only in microbial control, but also in virology, in general, if for no other reason than because of such diverse forms of insect viruses.

ACKNOWLEDGMENTS

I wish to thank Dr. M. Bergoin, Dr. A. Huger, Dr. D. W. Roberts, Mr. J. Robertson, and Dr. C. Vago for providing some of the slides that were used in this presentation.

REFERENCES CITED

Akutsu, K. 1968. The use of viruses for control of cabbage armyworm, *Mamestria brassicae* (Linnaeus), and common cabbageworm, *Pieris rapae crucivora* Boisduval. Proc. Joint U.S.-Japan Seminar Microbial Control Insect Pests, Fukuoka, April 21–23, 1967. pp. 43–49.

Aleksandrov, Y. N. 1965. Investigation of serological interrelations between polyhedron protein and nuclear polyhedrosis virus of some Lepidoptera. Mikrobiol. Z., Kiev. **27:** 80–84 (Russian with English summary).

Allen, G. E., Gregory, B. G. and Brazzel, J.R. 1967a. Integration of the *Heliothis* nuclear polyhedrosis virus into a biological control program on cotton. J. Econ. Entomol. **59:** 1333–1336.

Allen, G. E., Gregory, B. G. and Pate, T. L. 1967b. Field evaluation of a nuclear-polyhedrosis virus in the control of *Heliothis zea* and *Heliothis virescens* on cotton. J. Invertebr. Pathol. **9:** 40–42.

Anderson, L. D., Nakakihara, H. and Hall, I. M. 1963. Toxicity of new insecticides to corn earworms on sweet corn. J. Econ. Entomol. **56:** 40–42.

Aruga, H. and Hashimoto, Y. 1965. Interference between the UV-inactivated and active cytoplasmic-polyhedrosis viruses in the silkworm, *Bombyx mori* (Linnaeus). J. Sericult. Sci. Japan. **34:** 351–354.

Aruga, H., Hukuhara, T., Yoshitake, N. and Israngkul, A. 1961. Interference and latent infection in the cytoplasmic polyhedrosis of the silkworm, *Bombyx mori* (Linnaeus). J. Insect Pathol. **3:** 81–92.

Aruga, H., Watanabe, H. and Nagano, H. 1963a. Interference by the heat-inactivated virus on the active virus of the cytoplasmic polyhedrosis in the silkworm, *Bombyx mori* L. J. Sericult. Sci. Japan. **32:** 51–57.

Aruga, H., Yoshitake, N. and Watanabe, H. 1963b. Interference between cytoplasmic polyhedrosis viruses in *Bombyx mori* (Linnaeus). J. Insect Pathol. **5:** 1–10.

Aruga H., Yoshitake, N. and Watanabe, H. 1962. Interaction between inactivated virus and active virus of mid-gut polyhedrosis in the silkworm, *Bombyx mori* L. J. Sericult. Sci. Japan. **31:** 17–24.

Bellett, A. J. D. and Inman, R. B. 1967. Some properties of deoxyribonucleic acid preparations from *Chilo*, *Sericesthis* and *Tipula* iridescent viruses. J. Mol. Biol. **25:** 425–432.

Bird, F. T. 1959. Polyhedrosis and granulosis viruses causing single and double infections in the spruce budworm, *Choristoneura fumiferana* Clemens. J. Insect Pathol. **1:** 406–430.

Bird, F. T. 1969. Infection and mortality of spruce budworm, *Choristoneura fumiferana*, and forest tent caterpillar, *Malacosoma disstria*, caused by nuclear and cytoplasmic polyhedrosis viruses. Can. Entomol. **101:** 1269–1285.

Bird, F. T. and Burk, J. M. 1961. Artificially disseminated virus as a factor controlling the European spruce sawfly, *Diprion hercyniae* (Htg.) in the absence of introduced parasites. Can. Entomol. **93:** 228–238.

Bird, F. T. and Elgee, D. E. 1957. A virus disease and introduced parasites as factors controlling the European spruce sawfly, *Diprion hercyniae* (Htg.), central New Brunswick. Can. Entomol. **89:** 371–378.

Chapman, A. G. and Bell, R. A. 1967. Field tests with the nuclear polyhedrosis virus for control of *Heliothis* spp. in the southern High Plains of Texas, 1964. J. Econ. Entomol. **60:** 655–656.

Chapman, H. C., Clark, T. B., Woodard, D. B. and Kellen, W. R. 1966. Additional mosquito hosts of the mosquito iridescent virus. J. Invertebr. Pathol. **8:** 545–546.

Cunningham, J. C. and Longworth, J. F. 1968. The identification of some cyto-plasmic-polyhedrosis viruses. J. Invertebr. Pathol. **11:** 196–202.

David, W. A. L. 1965. The granulosis virus of *Pieris brassicae* L. in relation to natural limitation and biological control. Ann. App. Biol. **56:** 331–334.

Dikasova, E. T. 1964. Granulosis of the turnip moth and attempts to utilize this

disease in biological control of the pest. *In* "Biological Control of Agricultural and Forest Pests" (A. I. Cherepanov, ed.) Proc. Symp. 17–20 November, 1964. pp. 33–34. (Issled. po biol. metod. bor'by s vredit. sel'skogo i lesnogo khozyaistva (Dokl. k. simp. 17–20 noyabrya 1964g.)) Acad. Sci. U.S.S.R., Siberian Branch, Biol. Inst. (English transl. by Israel Program Sci. Transl., Jerusalem, 1969).

Doane, C. C. 1970. Primary pathogens and their role in the development of an epizootic in the gypsy moth. J. Invertebr. Pathol. **15:** 21–33.

Elmore, J. C. 1961. Control of the cabbage looper with a nuclear polyhedrosis virus disease. J. Econ. Entomol. **54:** 47–50.

Falcon, L. A., Kane, W. R. and Bethell, R. S. 1968. Preliminary evaluation of a granulosis virus for control of the codling moth. J. Econ. Entomol. **61:** 1208–1213.

Falcon, L. A., Leigh, T. F., van den Bosch, R., Black, G. H. and Burton, V. E. 1965. Insect diseases tested for control of cotton bollworm. Calif. Agr. **19:** 13–14.

Fernandez, A. T., Graham, H. M., Lukefahr, M. J., Bullock, H. R. and Hernandez, Jr., N. S. 1969. A field test comparing resistant varieties plus applications of polyhedral virus with insecticides for control of *Heliothis* spp. and other pests of cotton, 1967. J. Econ. Entomol. **62:** 173–177.

Fox, C. J. S. and Jaques, R. P. 1966. Preliminary observations on biological insecticides against imported cabbageworm. Can. J. Plant Sci. **46:** 497–499.

Genung, W. G. 1960. Comparison of insecticides, insect pathogens and insecticide-pathogen combinations for control of cabbage looper *Trichoplusia ni* (Hbn.) Florida Entomol. **43:** 65–68.

Gershenson, S. M. 1959. The variability of polyhedral viruses. Trans. First International Conf. Insect Pathol. and Biol. Control. Prague 1958. pp. 197–200 (In Russian with English Summary).

Getzin, L. W. 1962. The effectiveness of the polyhedrosis virus for the control of the cabbage looper, *Trichoplusia ni*. J. Econ. Entomol. **55:** 442–445.

Gilmore, J. E. 1965. Preliminary field evaluation of a noninclusion virus for control of the citrus red mite. J. Econ. Entomol. **58:** 1136–1140.

Gilmore, J. E. and Munger, F. 1965. Influence of population density on the incidence of a noninclusion virus disease of the citrus red mite in the laboratory. J. Invertebr. Pathol. **7:** 156–160.

Grison, P. 1960. Utilisation en forêt d'une préparation à base de virus spécifique contre *Thaumetopoea pityocampa* Schiff. Z. ang. Entomol. **47:** 24–31.

Harrap, K. A. and Robertson, J. S. 1968. A possible infection pathway in the development of a nuclear polyhedrosis virus. J. Gen. Virology **3:** 221–225.

Hofmaster, R. N. and Ditman, L. P. 1961. Utilization of a nuclear polyhedrosis virus to control the cabbage looper on cole crops in Virginia. J. Econ. Entomol. **54:** 921–923.

Huger, A. M. 1966. A virus disease of the Indian rhinoceros beetle, *Oryctes rhinoceros* (Linnaeus), caused by a new type of insect virus, *Rhabdionvirus oryctes* gen. n., sp. n. J. Invertebr. Pathol. **8:** 38–51.

Hukuhara, T. and Hashimoto, Y. 1966. Serological studies of the cytoplasmic- and nuclear-polyhedrosis viruses of the silkworm, *Bombyx mori*. J. Invertebr. Pathol. **8:** 234–239.

Ignoffo, C. M. 1964. Production and virulence of a nuclear-polyhedrosis virus from larvae of *Trichoplusia ni* (Hübner) reared on a semisynthetic diet. J. Insect Pathol. **6:** 318–326.

Ignoffo, C. M. 1968. Viruses—Living insecticides. Current Topics Microbiol. Immunol. **42:** 129–167.

Ignoffo, C. M., Chapman, A. J. and Martin, D. F. 1965. The nuclear-polyhedrosis virus of *Heliothis zea* (Boddie) and *Heliothis virescens* (Fabricius). III. Effectiveness of the virus against field populations of *Heliothis* on cotton, corn, and grain sorghum. J. Invertebr. Pathol. **7:** 227–235.

Jaques, R. P. 1964. The persistence of a nuclear-polyhedrosis virus in soil. J. Insect Pathol. **6:** 251–254.

Jaques, R. P. 1967. The persistence of a nuclear polyhedrosis virus in the habitat of the host insect, *Trichoplusia ni*. II. Polyhedra in Soil. Can. Entomol. **99:** 820–829.

Jaques, R. P. 1969. Leaching of the nuclear-polyhedrosis virus of *Trichoplusia ni* from soil. J. Invertebr. Pathol. **13:** 256–263.

Jaques, R. P. 1970a. Natural occurrence of viruses of the cabbage looper in field plots. Can. Entomol. **102:** 36–41.

Jaques, R. P. 1970b. Application of viruses to soil and foliage for control of the cabbage looper and imported cabbageworm. J. Invertebr. Pathol. **15:** 328–340.

Jaques, R. P. and Stultz, H. T. 1966. The influence of a virus disease and parasites on *Spilonota ocellana* in apple orchards. Can. Entomol. **98:** 1036–1045.

Kalmakoff, J. and Tremaine, J. H. 1968. Physicochemical properties of *Tipula* iridescent virus. J. Virol. **2:** 738–744.

Katagiri, K. 1969. Review on microbial control of insect pests in forests in Japan. Entomophaga **14:** 203–214.

Kaya, H. K. 1970. Toxic factor produced by a granulosis virus in armyworm larva: effect on *Apanteles militaris*. Science **168:** 251–253.

Koyama, R. and Katagiri, K. 1967. An application of nuclear and cytoplasmic polyhedrosis viruses against *Lymantria fumida* Butler (Lepidoptera: Lymantridae). Bull. Govt. Forest Expt. Sta. No. **207.** 10 pp.

Koyama, R. and Katagiri, K. 1968. Use of cytoplasmic polyhedrosis virus for the control of pine caterpillers, *Dendrolimus spectabilis* Butler (Lepidoptera: Lasiocampidae). Proc. Joint U.S.-Japan Seminar Microbial Control Insect Pest, Fukuoka, April 21–23, 1967. pp. 63–69.

Krywienczyk, J., Hayashi, Y. and Bird, F. T. 1969. Serological investigations of insect viruses. I. Comparison of three highly purified cytoplasmic-polyhedrosis viruses. J. Invertebr. Pathol. **13:** 114–119.

Luk'yanchikov, V. I. 1964. Granulosis of *Dendrolimus sibiricus* in nature and experiments in the conditions prevailing in the Tuva ASSR. *In* "Biological Control of Agricultural and Forest Pests" (A. I. Cherepanov, ed.) Proc. Symp. 17–20 November, 1964. pp. 51–53. (Issled. po biol. metod. bor'by s vredit. sel'skogo i lesnogo khozyaistva (Dokl. k. simp. 17–20 noyabrya 1964g.)) Acad. Sci. U.S.S.R., Siberian Branch, Biol. Inst. (English transl. by Israel Program Sci. Transl., Jerusalem, 1969).

Magnoler, A. 1968. Laboratory and field experiments on the effectiveness of purified and non-purified nuclear polyhedral virus of *Lymantria dispar* L. Entomophaga **13:** 335–344.

Marschall, K. J. 1970. Introduction of a new virus disease of the coconut rhinoceros beetle in Western Samoa. Nature **225:** 288–289.

McIntyre, T. and Dutky, S. R. 1961. Aerial application of virus for control of a pine sawfly, *Neodiprion pratti pratti*. J. Econ. Entomol. **54:** 809–810.

Meynadier, G., Fosset, J., Vago, C., Duthoit, J.-L. and Bres, N. 1968. Une virose à inclusions ovoïdes chez un Lépidoptère. Ann. Epiphyties **19:** 703–706.

Montoya, E. L., Ignoffo, C. M. and McGarr, R. L. 1966. A feeding stimulant to increase effectiveness of, and a field test with, a nuclear-polyhedrosis virus of *Heliothis*. J. Invertebr. Pathol. **8:** 320–324.

Morris, O. N. 1963. The natural and artificial control of the Douglas-fir tussock moth, *Orgyia pseudotsugata* McDunnough, by a nuclear polyhedrosis virus. J. Insect Pathol. **5:** 401–414.

Oatman, E. R., Hall, I. M., Arakawa, K. Y., Platner, G. R., Bascom, L. A. and Beegle, C. C. 1970. Control of the corn earworm on sweet corn in Southern California with a nuclear polyhedrosis virus and *Bacillus thuringiensis*. J. Econ. Entomol. **63:** 415–421.

Oho, N. 1966. A study on the cytoplasmic polyhedrosis virus of a fruit piercing moth, *Oraesia emarginata*. I. The details of discovery and its pathogenicity. Bul. Horticultural Res. Sta. Series A, No. **5.** pp. 165–178.

Oliver, A. O. 1964. Studies on the biological control of the fall webworm, *Hyphantria*

cunea, in Louisiana. J. Econ. Entomol. **57:** 314–318.
Ossowski, L. L. J. 1962. A polyhedrosis virus in wattle bagworm control. XI Intern. Congr. Entomol. Vienna 1960. **2:** 810–814.
Paillot, A. 1934. Un nouveau type de maladie à ultravirus chez les insectes. Compt. rend. acad. sci. **198:** 204–205.
Paillot, A. 1935. Nouvel ultravirus parasite d'*Agrotis segetum* provoquant une prolifération des tissus infectés. Compt. rend. acad. sci. **201:** 1062–1064.
Paillot, A. 1937. Nouveau type de pseudo-grasserie observé chez les chenilles d'*Euxoa segetum*. Compt. rend. acad. sci. **205:** 1264–1266.
Ponsen, M. b. and de Jong, D. J. 1964. On a nuclear and a cytoplasmic polyhedrosis of *Adoxophyes reticulana* Hb. Entomophaga **9:** 253–255.
Poltev, V. I. 1964. Experimental reproduction of granulosis in *Dendrolimus sibiricus*. *In* "Biological control of Agricultural and Forest Pests" (A. I. Cherepanov, ed.) Proc. Symp. 17–20 November, 1964. pp. 56–58. (Issled. po biol. metod. bor'by s vredit. sel'skogo i lesnogo khozyaistva (Dokl. k. simp. 17–20 noyabrya 1964g.)) Acad. Sci. U.S.S.R., Siberian Branch, Biol. Inst. (English transl. by Israel Program Sci. Transl., Jerusalem, 1969).
Putman, W. L. 1970. Occurrence and transmission of a virus disease of the European red mite, *Panonychus ulmi*. Can. Entomol. **102:** 305–321.
Rivers, C. F. 1964. The use of a polyhedral virus disease in the control of the pine sawfly *Neodiprion sertifer* Geoffr. in north-west Scotland. Colloq. Intern. Path. Insectes, Lutte Microbiol., Paris 1962. Entomophaga, Mém. Hors Sér. No. **2.** pp. 477–480.
Roberts, D. W. and Granados, R. R. 1968. A poxlike virus from *Amsacta moorei* Lepidoptera: Arctiidae). J. Invertebr. Pathol. **12:** 141–143.
Rollinson, W. D. Lewis, F. B. and Waters, W. E. 1965. The successful use of a nuclear-polyhedrosis virus against the gypsy moth. J. Invertebr. Pathol. **7:** 515–517.
Seecof, R. 1968. The sigma virus infection of *Drosophila melanogaster*. Current Topics Microbiol. Immunol. **42:** 59–93.
Semel, M. 1961. The efficiency of a polyhedrosis virus and *Bacillus thuringiensis* for control of the cabbage looper on cauliflower. J. Econ. Entomol. **54:** 698–701.
Shaw, J. G., Chambers, D. L. and Tashiro, H. 1968a. Introducing and establishing the noninclusion virus of the citrus red mite in citrus groves. J. Econ. Entomol. **61:** 1352–1355.
Shaw, J. G., Tashiro, H. and Dietrick, E. J. 1968b. Infection of the citrus red mite with virus in central and southern California. J. Econ. Entomol. **61:** 1492–1495.
Sidor, C'. 1965. The susceptibility of *Thaumetopoea pityocampa* (Schiff.) to a specific polyhedral virus disease and to the cytoplasmic polyhedral virus of *Arctia caja* (L.). Proc. 12th Intern. Congr. Entomol., London 1964. pp. 743–744.
Sidor, C'. and Nikolić, V. 1966. Viroze diprion vrstan Jugoslaviji injihova primena za suzbijanje pagusenica na terenu. I. Polidarno virozno oboljenje *Neodiprion sertifer* Geoffr. Ann. Sci. Work Fac. Agr. Novi Sad. No. **10.** pp. 37–53.
Smirnoff, W. A. 1961. A virus disease of *Neodiprion swainei* Middleton. J. Insect Pathol. **3:** 29–46.
Smirnoff, W. A. 1964. Study of the virus disease of *Neodiprion swainei* Midd. and results of its aerial dispersion. Entomophaga Mem. Hors Sér. Numéro 2. pp. 475–476.
Smirnoff, W. A., Fettes, J. J. and Haliburton, W. 1962. A virus disease of Swaine's Jack pine sawfly, *Neodiprion swainei* Midd. sprayed from an aircraft. Can. Entomol. **94:** 477–486.
Stairs, G. R. 1964a. Selection of strain of insect granulosis virus producing only cubic inclusion bodies. Virology **24:** 520–521.
Stairs, G. R. 1964b. Dissemination of nuclear polyhedrosis virus against the forest tent caterpillar, *Malacosoma disstria* (Hübner) (Lepidoptera: Lasiocampidae). Can. Entomol. **96:** 1017–1020.
Stairs, G. R. 1965. Artificial initiation of virus epizootics in forest tent caterpillar

populations. Can. Entomol. **97:** 1059–1062.
Stairs, G. R. and Bird, F. T. 1962. Dissemination of viruses against the spruce budworm *Choristoneura fumiferana* (Clemens). Can. Entomol. **94:** 966–969.
Stairs, G. R., Parrish, W. B., Briggs, J. D. and Allietta, M. 1966. Fine structure of a granulosis virus of the codling moth. Virology **30:** 583–584.
Stelzer, M. J. 1965. Susceptibility of the great basin tent caterpillar, *Malacosoma fragile* (Stretch), to a nuclear-polyhedrosis virus and *Bacillus thuringiensis* Berliner. J. Invertebr. Pathol. **7:** 122–125.
Stelzer, M. J. 1967. Control of a tent caterpillar, *Malacosoma fragile incurva*, with an aerial application of a nuclear-polyhedrosis virus and *Bacillus thuringiensis*. J. Econ. Entomol. **60:** 38–41.
Summers, M. D. 1969. Apparent in vivo pathway of granulosis virus invasion and infection. J. Virology **4:** 188–190.
Tanada, Y. 1956. Some factors affecting the susceptibility of the armyworm to virus infections. J. Econ. Entomol. **49:** 52–57.
Tanada, Y. 1959. Synergism between two viruses of the armyworm, *Pseudaletia unipuncta* (Haworth) (Lepidoptera, Noctuidae). J. Insect Pathol. **1:** 215–231.
Tanada, Y. 1961. The epizootiology of virus diseases in field populations of the armyworm, *Pseudaletia unipuncta* (Haworth). J. Insect Pathol. **3:** 310–323.
Tanada, Y. and Hukuhara, T. 1968. A nonsynergistic strain of a granulosis virus of the armyworm, *Pseudaletia unipuncta*. J. Invertebr. Pathol. **12:** 263–268.
Tanada, Y. and Hukuhara, T. 1971. Enhanced infection of a nuclear-polyhedrosis virus in larvae of the armyworm, *Pseudaletia unipuncta*, by a factor in the capsule of a granulosis virus. J. Invertebr. Pathol. **17:** 116–126.
Tanada, Y., Hukuhara, T. and Chang, G. Y. 1969. A strain of nuclear-polyhedrosis virus causing extensive cellular hypertrophy. J. Invertebr. Pathol. **13:** 394–409.
Tanada, Y. and Leutenegger, R. 1970. Multiplication of a granulosis virus in larval midgut cells of *Trichoplusia ni* and possible pathways of invasion into the hemocoel. J. Ultrastructure Res. **30:** 589–600.
Tanada, Y. and Reiner, C. 1962. The use of pathogens in the control of the corn earworm, *Heliothis zea* (Boddie). J. Insect Pathol. **4:** 139–154.
Tanada, Y. and Watanabe, H. 1971. Disc electrophoretic patterns of hemolymph proteins of larvae of the armyworm, *Pseudaletia unipuncta*, infected with a nuclear-polyhedrosis and a granulosis virus. J. Invertebr. Pathol. **17:** 127–129.
Thompson, C. G. and Steinhaus, E. A. 1950. Further tests using a polyhedrosis virus to control the alfalfa caterpillar. Hilgardia **19:** 411–445.
Vago, C. 1963. A new type of insect virus. J. Insect Pathol. **5:** 275–276.
Vago, C., Amargièr, A., Hurpin, B., Meynadier, G. and Duthoit, J.-L. 1968. Virose à fuseaux d'un Scarabéide d'Amérique du Sud. Entomophaga **13:** 373–376.
Vago, C., Meynadier, G. and Duthoit, J.-L. 1964. Étude d'un nouveau type de maladie à virus chez les Lépidoptères. Ann. Épiphyties **15:** 473–477.
Wilson, F. 1960. The effectiveness of a granulosis virus applied to field populations of *Pieris rapae* (Lepidoptera). Australian J. Agr. Res. **11:** 485–497.
Wolfenbarger, D. A. 1964. Paraffinic and naphthenic oil fraction in combination with DDT and a *Heliothis* virus for corn earworm control. J. Econ. Entomol. **57:** 732–735.
Wolfenbarger, D. A. 1965. Polyhedrosis-virus-surfactant and -insecticide combinations, and *Bacillus thuringiensis*-surfactant combinations, for cabbage looper control. J. Invertebr. Pathol. **7:** 33–38.
Woodall, K. L. and Ditman, L. P. 1967. Control of the cabbage looper and corn earworm with nuclear polyhedrosis viruses. J. Econ. Entomol. **60:** 1558–1561.
Woodard, D. B. and Chapman, H. C. 1968. Laboratory studies with the mosquito iridescent virus (MIV). J. Invertebr. Pathol. **11:** 296–301.
Xeros, N. 1954. A second virus disease of the leatherjacket, *Tipula paludosa*. Nature **174:** 562.
Young, J. R. and Hamm, J. J. 1966. Nuclear-polyhedrosis viruses in control of corn earworm and fall armyworm in sweet corn. J. Econ. Entomol. **59:** 382–384.